Amendments to the Title, Specification and Abstract:

The amendments made to the specification are merely to correct typographical errors and indicate trademarks where appropriate.

Amendments made to the Title and Abstract were made to more accurately reflect the currently claimed invention.

In accordance with 37 C.F.R. §1.121(b)(1)(iii), attached hereto as Exhibit 1, is a marked up version of the changes made to the Title, specification, and Abstract by this Amendment. Accordingly, entry of and consideration of the amendments to the subject application are respectfully requested.

Amendments to the claims:

The amendment does not involve new matter. Entry of these changes is requested.

RESTRICTION REQUIREMENT

Applicants acknowledge that the restriction requirement is deemed proper and final.

PRIORITY

In Paragraph 3 of the Office Action, the Examiner has taken the position that the priority date of the claimed methods is July 27, 1991, the filing date of the parent application, U.S. Serial No. 07/722,101.

Applicants respectfully disagree. The claimed methods are entitled to both the priority dates of March 26, 1990 and July 2, 1990 in connection with parent applications U.S. Serial No. 07/498,949 and U.S. Serial No. 07/547,980, respectively.

Support may be found as follows:

Support for amended claim 87 is found in U.S. Serial No. 07/498,949 at page 4, lines 10-19, page 7, lines 17-35, page 8, lines 1-11, page 11, lines 4-19 and page 13, lines 7-15 and in U.S. Serial No. 07/547,980, at page 4, lines 21-35, page 7, lines 29-35, page 8, lines 1-23, page 11, lines 16-31 and page 13, lines 19-24.

Support for new claim 89 is found in U.S. Serial No. 07/498,949 at page 7, lines 17-35 and page 8, lines 1-11; in U.S. Serial No. 07/547,980, at page 7, lines 29-35 and page 8, lines 1-23; and in U.S. Serial Nos. 08/219,200 and 09/666,267 at page 19, lines 11-12.

Support for amended claim 90 is found in U.S. Serial No. 07/498,949 at page 4, lines 10-19, page 7, lines 17-35, page 8, lines 1-11, page 11, lines 4-19 and page 13, lines 7-15 and in U.S. Serial No. 07/547,980, at page 4, lines 21-35, page 7, lines 29-35, page 8, lines 1-23, page 11, lines 16-31 and page 13, lines 19-24.

Support for new claim 91 is found in U.S. Serial No. 07/498,949 at page 7, lines 17-35 and page 8, lines 1-11; in U.S. Serial No. 07/547,980, at page 7, lines 29-35 and page 8, lines 1-23; and in U.S. Serial Nos. 08/219,200 and 09/666,267 at page 19, lines 11-12.

Support for new claim 92 is found in U.S. Serial Nos. 08/219,200 and 09/666,267 at page 58, lines 26-31.

Support for new claim 93 is found in U.S. Serial No. 07/498,949 at page 11, lines 4-19; U.S. Serial No. 07/547,980, at page 11, lines 16-31; and in U.S. Serial Nos. 08/219,200 and 09/666,267 at page 19-20.

TITLE OF THE INVENTION

In Paragraph 3 of the Office Action, the Patent Office suggests amending the title of the invention to reflect the currently pending claims. Applicants have done so hereinabove.

ABSTRACT OF THE DISCLOSURE

In paragraph 4, the Patent Office suggests amending the abstract of the disclosure to reflect the pending claims. In response, Applicants have done so hereinabove.

FORMAL FIGURES

In paragraph 5, the Patent Office stated that the formal drawings and photographs that were submitted fail to comply with 37 C.F.R. §1.84. In response, applicants provide a Petition Under 37 C.F.R. §1.182 requesting that the accepted formal figures in the parent application be used in this case (annexed herewith as Exhibit 2).

TYPOGRAPHICAL ERRORS AND TRADEMARKS

In Paragraph 6 of the Office Action, the Patent Office suggests correcting all spelling errors and indicating trademarks, where appropriate. In response, Applicants have made the corrections hereinabove.

SEQUENCE COMPLIANCE

In paragraph 7 of the Office Action, the Examiner has taken the position that the application has failed to comply with 37 C.F.R. §1.821-1.825.

A diskette containing the B7 amino acid sequence for amino acids 1-216 as published on page 2717 by Freeman et al., 1989 (SEQ. ID NO. 8) is enclosed. A paper copy of all of the Sequence Listings (i.e., SEQ ID Nos. 1-8) is also enclosed (Exhibit 3).

For convenience, the diskette is inclusive of originally filed SEQ. ID. Nos. 1-7, and SEQ. ID. NO. 8 as requested by the Examiner. A Declaration stating that the electronic copy of the Sequence Listings is identical to the paper copy is also enclosed (Exhibit 4).

I. REJECTIONS UNDER 35 U.S.C. §112, FIRST PARAGRAPH

In paragraphs 8-9 of the Office Action, the Patent Office rejected Claims 87-88 under 35 U.S.C. §112, first paragraph, stating that "the specification does not contain a written description of the claimed invention, in that the disclosure does not reasonably convey to one skilled in the relevant art that the inventor(s) had possession of the claimed invention at the time the application was filed." Specifically, the Patent Office alleged that the specification does not provide sufficient written description for the particular steps in claim 87a-e and claim 88.

Applicants respectfully disagree. However, in order to further the prosecution of the subject application, applicants have amended the claims to recite methods for producing antibodies in accordance with the Examiner's suggestion.

Although the rejection is moot since the claims have been amended to more closely track the exact language of the specification and in accordance with the Examiner's suggestion, for the record, Applicants state that claim 87 as set forth in Applicants' response dated December 20, 2000, was described sufficiently under 35 USC §112 first and second paragraph. For example, support for claim 87 steps (a)-(e) is as follows:

- step (a) which describes an assay for identifying B cells that bind CD28, is supported by the CD28-mediated cell adhesion assay shown in the subject application as originally filed at Figure 3 and page 34, lines 5-35, page 35 and page 36, lines 1-25;
- step (b) which describes isolating and purifying proteins mediating B cell binding with CD28 i.e., B7 or CD28 or fragments or derivative thereof, is supported in the subject application as originally filed at Figure 10 and page 18, lines 17-34 and page 57, lines 14-33.
- step (c) which describes immunizing an animal with an antigenic portion of the purified protein of step (b) i.e., a B7 or CD28 immunogen such as B7Ig, is supported in the subject application as originally filed at page 19, lines 9-14, line 35 and page 20, line 1.
- step (d) which describes harvesting antibodies produced by the previous steps, is supported in the subject application as originally filed at page 20, lines 5-16.
- and step (e) which describes screening the antibodies produced for those that inhibit CD28 binding to B cells, is supported in the subject application as originally filed at page 23, lines 1-18.

In paragraphs 10-11 of the Office Action, the Patent Office rejected Claims 87-88 under 35 U.S.C. §112, first paragraph, stating that "the specification does not contain a written description of the claimed invention, in that the disclosure does not reasonably convey to

one skilled in the relevant art that the inventor(s) had possession of the claimed invention at the time the application was filed." Specifically, the Patent Office alleged that the term B7 antigen is too broad and should include the sequence for a B7 as disclosed by Freeman et al.

Applicants respectfully disagree. However, in order to further the prosecution of the subject application, applicants have amended the claims to recite methods for producing antibodies that react with B7 having the sequence in SEQ ID. NO. 8 in accordance with the Examiner's suggestion. Applicants have amended the specification at page 11, line 17 as set forth above. A signed Declaration pursuant to MPEP § 608.01(p) is also enclosed (Exhibit S).

In paragraph 12 of the Office Action, the Patent Office is requesting a sequence listing including the sequence information regarding B7 as disclosed by Freeman et al. In response, applicants provide a new sequence listing including the B7 sequence of Freeman et al. (SEQ ID NO. 8).

Additionally, the Examiner is requiring applicants to amend the specification to include the date of the biological deposit and the current address of the American Type Culture Collection (ATCC). The amendment of the specification at paragraph at page 13, lines 9-10, now includes the deposit date of the B7 Ig cDNA and updated address and does not involve new matter. Entry of the amendment is requested.

Applicants respectfully contend that the B7Ig (ATCC No. 68627) was deposited with the American Type Culture Collection, pursuant to the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure with the patent culture depository of the ATCC, 10801 University Blvd., Manassas, Virginia 20110-2209 U.S.A. The deposit was made on May 31, 1991.

Applicants maintain that during the pendency of the subject application, access to the ATCC deposits will be afforded to one determined by the Patent Commissioner to be entitled thereto under 35 U.S.C. §1.14 and §122, and all restrictions on the availability to the public of the cDNA deposited under the ATCC Accession No. listed above will be irrevocably removed upon the issuance of a patent from the subject application. Furthermore, the above deposits will be maintained by the ATCC for a period of 30 years from the date of deposit or at least 5 years after the last request for a sample of the deposited material, whichever is longer. Where the ATCC cannot furnish samples of the above deposits for any reason, applicants shall make a replacement deposit, of the material which was originally deposited, within three months of receiving notification that the ATCC cannot furnish samples.

In paragraph 13 of the Office Action, the Patent Office rejected claims 87-88 under 35 U.S.C. §112, first paragraph, because the specification while being enabling for methods of producing monoclonal antibodies reactive with B7Ig fusion proteins or B7 antigen positive cells does not provide enablement for any methods for producing antibodies to B7 including the particular steps of claim 87a-e and claim 88 as currently recited.

In response, applicants have amended the claims as suggested by the Examiner. The changes to the claims should render moot the rejection.

PRIOR ART

In paragraphs 14-15 of the Office Action, the Patent Office is rejecting the pending claims under 35 U.S.C. §103a in view of Ledbetter et al. (U.S. Patent No. 5,182,368) in view of Linsley et al. (PNAS 87:5031-5015 (1990), and Freeman et al. (J. Immunol. 143:2714-2722 (1989)).

The data in Linsley et al. (published July 1990) is applicants' own work and described in the parent application, U.S. Serial No. 07/547,980, filed July 2, 1990 (the parent '980 application, Example at pages 15-30) and U.S. Serial No. 07/498,949 filed March 26, 1990 (the parent '949 application, Example at pages 13-28). Specifically, the subject matter in Linsley et al. (1990) corresponds to and U.S. Serial No. 07/498,949 filed March 26, 1990 and U.S. Serial No. 07/547,980 as shown in the following:

- Linsley et al. (1990) at Table 1 (page 5034) corresponds to the parent '949 and '980 applications at Table 1 (specification at page 24 and 26, respectively);
- Linsley et al. (1990) at Figures 1-5 corresponds to Figures 1-5 of the parent '949 and '980 applications;
- Linsley et al. (1990) at page 5031, left column, paragraphs 2-5 corresponds to the parent '949 and '980 applications in "The "Background" section at pages 1-2 and 1-2, respectively;
- Linsley et al. (1990) at Page 5032, left column, paragraphs 3-5 corresponds to the section entitled "Cell Adhesion Assay for a CD28 Ligand" of parent '949 application, at page 17, lines 33-35, pages 18-19 and page 20, lines 1-21, and '980 application at page 19, lines 18-35, pages 20-21 and page 22, lines 1-7, respectively;
- Linsley et al. (1990) at page 5032, right column, second paragraph and page 5033, left column corresponds to the section entitled "The CD28 Ligand is a B Cell Activation Marker" of the parent '949 application at page 20, lines 23-35 and page 21, lines 1-22 and in '980 application at page 22, lines 9-35 and page 23, lines 1-8;
- Linsley et al. (1990) at page 5033, left column, paragraph 3 and right column, paragraphs 1-3 corresponds to the section entitled "CD28-Mediated Adhesion is Specifically Blocked by a MAb (BB-1) to a B Cell Activation Antigen" of the parent '949 application at page 21, lines 24-35, page 22, page 23, lines 1-6 and in '980 application at page 23, lines 10-35 and page 24, lines 1-27;
- Linsley et al. (1990) at page 5033, right column, last paragraph and pag 5034, left column, first paragraph corresponds to the section entitled "Binding of MAb BB-1

by Different Cells Correlates With CD28-Specific Adhesion" of the parent '949 application at page 23, lines 8-34, page 24, lines 1-39 and in '980 application at page 24, lines 29-35, page 25 and page 26, lines 1-35;

- Linsley et al. (1990) at page 5034, left column, second paragraph and right column, first paragraph in corresponds to the section entitled "COS Cells Expressing the B7/BB-1 Antigen Adhere Specifically to CD28" of the parent '949 application at page 24, lines 40-47, page 25 and in the '980 application at page 26, lines 38-47 and page 27, lines 1-31;
- Linsley et al. (1990) at the "Discussion" section at page 5034, right column, paragraphs 2-5 and page 5035, left column corresponds to the parent '949 application at pages 26-28 and in the '980 application in the section at page 27, lines 34-35 and page 28-30.

The subject matter of claims 87-88 is fully disclosed at least as early as March 26, 1990 in application U.S. Serial No. 07/498,949. Accordingly, the claimed invention is entitled to the benefit of the March 26, 1990 filing date and therefore Linsley et al. (July 1990) is not prior art. Without Linsley, the combination fails.

CONCLUSION

Applicants submit that the foregoing amendments put this application in condition for allowance, notice of which is respectfully requested. If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone her at the number provided below.

No fee, other than the extension fee, is deemed necessary in connection with the filing of this Amendment. If any fee is necessary, the Patent Office is authorized to charge any additional fee to Deposit Account No. 50-0306.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE IN THE SPECIFICATION

In the Title:

In accordance with 37 C.F.R. §§1.121(b)(1)(i)-(ii), please delete the title beginning on page 1 of the application and replace it with the following:

-- [LIGAND FOR CD28 RECEPTOR ON B CELLS AND] METHODS FOR GENERATING AND IDENTIFYING ANTIBODIES DIRECTED AGAINST A B7 --

In the Specification:

Please the amend the specification as follows:

Please replace the paragraph beginning at page 9, lines 21-25, with the following rewritten paragraph:

--Figure 7a is graphs showing the effects of DR7-primed $CD4^+CD45RO^+$ T_h cells on differentiation of B cells into IgM [immunoglobulin] secreting SKW B cells, as described in Example 2, [infra] *infra*.[(7a: IgM production by SKW B cells; 7b: IgG production by CESS B cells).]

Figure 7b is a graph showing the effects of DR7-primed $CD4^+CD45RO^+$ T_h cells on differentiation of B cells into IgG secreting CESS B cells, as described in Example 2, *infra*.--

Please replace the paragraph beginning at page 9, lines 28-30, with the following rewritten paragraph:

--Figure 8a is a graph[s] showing the effect of anti-CD28 and anti-B7 mAbs on the T_h-induced production of IgM [immunoglobulin] by B cells as described in Example 2, [infra] *infra.* [(8a: IgM production, 8b: IgG production).]

Figure 8b is a graph showing the effect of anti-CD28 and anti-B7 mAbs on the T_h-induced production of IgG by B cells as described in Example 2, *infra*.--

Please replace the paragraph beginning at page 9, lines 32-35, with the following rewritten paragraph:

-- Figure 9a is a diagrammatic representation of B7Ig [(9a) and CD28Ig (9b)] protein fusion constructs as described in Example 3, [infra] *infra* (dark shaded regions = oncostatin M; unshaded regions = B7 [and CD28], stippled regions = human Ig Cγ1).

Figure 9b is a diagrammatic representation of CD28Ig protein fusion constructs as described in Example 3, *infra* (dark shaded regions = oncostatin M; unshaded regions = CD28, stippled regions = human Ig Cγ1).—

Please replace the paragraph at page 11, line 8, with the following rewritten paragraph:

-- Recently, Freeman et al., (J. Immunol. 143 (8): 2714-2722 (1989)) isolated and sequenced a cDNA clone encoding a B cell activation antigen recognized by monoclonal antibody (mAb) B7 (Freedman et al., J. Immunol. 139:3260 (1987)). COS cells transfected with this cDNA were shown to stain by both mAb B7 and mAb BB-1 (Clark et al., Human Immunology 16:100-113 (1986), and Yokochi et al., (1981), supra; Freeman et al., (1989) supra; and Freedman et al., (1987), supra)). The ligand for CD28 was identified by the experiments described herein, as the B7/BB-1 antigen isolated by Freeman et al., wherein the predicted amino acid sequence of amino acid 1-216 are:

Gly Leu Ser His Phe Cys Ser Gly Val Ile His Val Thr Lys Glu Val
1 5 10 15

Lys Glu Val Ala Thr Leu Ser Cys Gly His Asn Val Ser Val Glu Glu
20 25 30

Leu Ala Gln Thr Arg Ile Tyr Trp Gln Lys Glu Lys Lys Met Val Leu
35 40 45

Thr Met Met Ser Gly Asp Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg
50 55 60

Thr Ile Phe Asp Ile Thr Asn Asn Leu Ser Ile Val Ile Leu Ala Leu
65 70 75 80

Arg Pro Ser Asp Glu Gly Thr Tyr Glu Cys Val Val Leu Lys Tyr Glu
85 90 95

Lys Asp Ala Phe Lys Arg Glu His Leu Ala Glu Val Thr Leu Ser Val
100 105 110

Lys Ala Asp Phe Pro Thr Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr
115 120 125

Ser Asn Ile Arg Arg Ile Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu
130 135 140

Pro His Leu Ser Trp Leu Glu Asn Gly Glu Glu Leu Asn Ala Ile Asn
145 150 155 160

Thr Thr Val Ser Gln Asp Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser
165 170 175

Lys Leu Asp Phe Asn Met Thr Thr Asn His Ser Phe Met Cys Leu Ile
180 185 190

Lys Tyr Gly His Leu Arg Val Asn Gln Thr Phe Asn Trp Asn Thr Thr
195 200 205

Lys Gln Glu His Phe Pro Asp Asn
210 215

(Freedman et al., and Freeman et al., supra, both of which are incorporated by reference herein). --

On page 30, please replace the first full paragraph with:

-- In a preferred embodiment, DNA encoding the amino acid sequence corresponding to the extracellular domain of the B7 antigen, containing amino acids from about position 1 to about position 215, is joined to DNA encoding the amino acid sequences corresponding to the hinge, CH2 and CH3 regions of human Ig Cγ1, using PCR, to form a construct that is expressed as B7Ig fusion protein. DNA encoding the amino acid sequence corresponding to the B7Ig fusion protein has been deposited with the American Type Culture Collection (ATCC) [in Rockville, Maryland] at 10801 University Blvd., Manassas, Virginia 20110-2209 U.S.A., under the Budapest Treaty on May 31, 1991 and accorded accession number 68627.--

On page 30, line 14, please replace the paragraph beginning "Cell Culture" with the following paragraph:

-- Cell Culture. T51, 1A2, 5E1, Daudi, Raji, Jijoye, CEM, Jurkat, HSB2, THP-1 and HL60 cells (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA) were cultured in complete RPMI™ medium (RPMI™ containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin. Dhfr-deficient Chinese hamster ovary (CHO) cells (Urlaub and Chasin, Proc. Natl. Acad Sci., 77:4216-4220 (1980)) were cultured in Maintenance Medium (Ham's F12 [m]Medium™ (GIBCO, Grand Island, NY) supplemented with 10% FBS, 0.15 mM L-proline, 100 U/ml penicillin and 100 µg/ml streptomycin). Dhfr-positive transfectants were selected and cultured in Selective Medium (DMEM™, supplemented with 10% FBS, 0.15 mM L-proline, 100 U/ml penicillin and 100 µg/ml streptomycin).- -

On page 30, line 28, please replace the paragraph bridging pages 30 and 31, and beginning "Spleen B cells", with the following paragraph:

-- Spleen B cells were purified from Balb/c mice by treatment of total spleen cells with an anti-Thy 1.2 mAb (30H12) (Ledbetter and Herzenberg, Immunol. Rev. 47:361-389 (1979)) and baby rabbit complement. The resulting preparations contained approximately 85% B cells, as judged by FACS® analysis following staining with fluorescein

isothiothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin (TAGO). These cells were activated by treatment for 72 hrs with E. coli lipopolysaccharide (LPS, List Biological Laboratories, Campbell, CA) at 10 μg/ml in complete RPMI™.--

On page 31, line 5, please replace the paragraph "Monoclonal Antibodies" with the following:

-- Monoclonal Antibodies. Monoclonal antibody (mAb) 9.3 (anti-CD28) (ATCC No. HB 10271, Hansen et al., Immunogenetics 10:247-260 (1980)) was purified from ascites before use. mAb 9.3 $F(ab')_2$ fragments were prepared as described by Parham, in J. Immunol. 131:2895-2902 (1983). Briefly, purified mAb 9.3 was digested with pepsin at pH 4.1 for 75 min. followed by passage over protein A SEPHAROSE™ (beaded agarose) to remove undigested mAb. A number of mAbs to B cell-associated antigens were screened for their abilities to inhibit CD28-mediated adhesion. mAbs 60.3 (CD18); 1F5 (CD20); G29-5 (CD21); G28-7, HD39, and HD6 (CD22); HD50 (CD23); KB61 (CD32); G28-1 (CD37); G28-10 (CD39); G28-5 (CD40); HERMES1 (CD44); 9.4 (CD45); LB-2 (CD54) and 72F3 (CD71) have been previously described and characterized in International Conferences on Human Leukocyte Differentiation Antigens I-III (Bernard et al., Eds., Leukocyte Typing, Springer-Verlag, New York (1984); Reinherz et al., Eds., Leukocyte Typing II Vol. 2 New York (1986); and McMichael et al., Eds., Leukocyte Typing III Oxford Univ. Press, New York, (1987)). These mAbs were purified before use by protein A SEPHAROSE™ (beaded agarose) chromatography or by salt precipitation and in exchange chromatography. δTA401 (Kuritani and Cooper, J. Exp. Med. 155:839-848 (1982)) (Anti-IgD); 2C3 (Clark et al., (1986), supra) (anti-IgM); Nambl, H1DE, P10.1, W6/32 (Clark et al., (1986) supra; and Gilliland et al., Human Immunology 25:269-289 (1989), anti-human class I); and HB10A (Clark et al., (1986), supra, anti-MHC class II) were also purified before use. mAbs B43 (CD19); BL-40 (CD72); AD2, 1E9.28.1, and 7G2.2.11 (CD73); EBU-141, LN1 (CDw75); CRIS-1 (CD-76); 424/4A11, 424/3D9 (CD77) Leu 21, Ba, 1588, LO-panB-1, FN1, and FN4 (CDw78); and M9, G28-10, HuLym10, 2-7, F2B2.6, 121, L26, HD77, NU-B1, BLAST-1, BB-1. anti-BL7, anti-

HC2, and L23 were used as coded samples provided to participants in the Fourth International Conference on Human Leukocyte Differentiation Antigens (Knapp, Ed., Leukocyte Typing IV, Oxford Univ. Press, New York (1990). These were used in ascites form. mAbs BB-1 and LB-1 (Yokochi et al., (1981), supra) were also purified from ascites before use. Anti-integrin receptor mAbs P3E3, P4C2, P4G9 (Wayner et al., J. Cell. Biol. 109:1321-1330 (1989)) were used as hybridoma culture supernatants.--

On page 32, line 10, please replace the paragraph beginning "Immunostaining Techniques." with the following:

-- Immunostaining Techniques. For indirect immunofluorescence, cells were incubated with mAbs at 10 μg/ml in complete RPMI™ for 1 hr at 4°C. mAb binding was detected with a FITC-conjugated goat anti-mouse immunoglobulin second step reagent. For direct binding experiments, mAbs 9.3 and BB-1 were directly conjugated with FITC as described by Goding in Monoclonal Antibodies: Principles and Practices Academic Press, Orlando, FL (1983), and were added at saturating concentrations in complete RPMI™ for 1 hr at 4°C. Non-specific binding of FITC-conjugated mAbs was measured by adding the FITC conjugate following antigen pre-blocking (20-30 min at 4°C) with unlabeled mAb 9.3 or BB-1. Immunohistological detection of adherent lymphoblastoid cells was achieved using the horseradish peroxidase (HRP) method described by Hellstrom et al., J. Immunol. 127:157-160 (1981).--

On page 34, line 15, please replace the two paragraphs beginning "CD28-Mediated Adhesion Assay" and "Labeled cells" with the following two paragraphs:

-- CD28-Mediated Adhesion Assay. Cells to be tested for adhesion were labeled with ^{51}Cr (0.2-1 mCi) to specific activities of 0.2-2 cpm/cell. A mouse mAb having irrelevant specificity, mAb W1, directed against human breast carcinoma-associated mucin, (Linsley et al., Cancer Res. 46:5444-5450 (1986)), was added to the labeling reaction to a final concentration of 100 μg/ml to saturate Fc receptors. Labeled and washed cells were preincubated in complete RPMI™ containing 10 μg/ml of mAb W1, and unless otherwise

indicated, 10 mM EDTA. mAb 9.3 or mAb 9.3 F(ab')$_2$ was added to some samples at 10 µg/ml, for approximately 1 hr at 23^{o}C.

Labeled cells (1-10 x 10^6/well in a volume of 0.2 ml complete RPMI™, containing EDTA and mAbs, where indicated) were then added to the CHO monolayers. Adhesion was initiated by centrifugation in a plate carrier (1,000 rpm, in a Sorvall HB1000 rotor, approximately 210 X g) for 3 min at 4^{o}C. Plates were then incubated at 37^{o}C for 1 hr. Reactions were terminated by aspirating unbound cells and washing five times with cold, complete RPMI™. Monolayers were solubilized by addition of 0.5 N NaOH, and radioactivity was measured in a gamma counter. For most experiments, numbers of bound cells were calculated by dividing total bound radioactivity (cpm) by the specific activity (cpm/cell) of labeled cells. When COS cells were used, their viability at the end of the experiment was generally less than 50%, so specific activity calculations were less accurate. Therefore, for COS cells results are expressed as cpm bound.--

On page 35, line 22, please replace the paragraph beginning "The effects of divalent cation depletion" with the following:

-- The effects of divalent cation depletion on T51 cell adhesion to CD28$^+$ and CD28$^-$ CHO cells were examined. Preliminary experiments showed that EDTA treatment caused loss of CHO cells during washing, so the CHO cell monolayers were fixed with paraformaldehyde prior to EDTA treatment. Fixation did not significantly affect CD28-mediated adhesion by T51 cells either in the presence or absence of mAb 9.3. Monolayers of CD28$^+$ and CD28$^-$ CHO cells (1 to 1.2 X 10^5/cm^2 in 48 well plastic dishes) were fixed in 0.5% paraformaldehyde for 20 min at 23^{o}C, washed and blocked in [C]complete RPMI™ for 1 hr, then pre-incubated with or without mAb 9.3 or mAb 9.3 F(ab')$_2$ at 10 µg/ml in [C]complete RPMI™ for 1 hr at 37^{o}C. T51 cells were labeled with ^{51}Cr, preincubated with or without 10 mM EDTA, added to CHO cells and cellular adhesion was measured. The results are presented in Figure 1. Mean and standard deviation (error bars) are shown for three replicate determinations.--

On page 45, line 9, please replace the paragraph beginning "Culture medium" with the following:

-- Culture medium. Complete culture medium (CM) consisted of RPMI™ 1640 (Irvine Scientific, Santa Ana, CA) supplemented with 100 U/ml of penicillin G, 100 µg/ml of streptomycin, 2 mM L-glutamine, 5 X 10^{-5} M 2-ME, and 10% FBS (Irvine Scientific).--

On page 55, line 31 through page 56, line 30, please replace the two paragraphs beginning "Cell Culture and Transfections" and "CHO cells expressing CD28," with the following paragraphs:

-- Cell Culture and Transfections. COS (monkey kidney cells) were transfected with expression plasmids using a modification of the protocol of Seed and Aruffo (Proc. Natl. Acad. Sci. 84:3365 (1987)), incorporated by reference herein. Cells were seeded at 10^6 per 10 cm diameter culture dish 18-24 h before transfection. Plasmid DNA was added (approximately 15 µg/dish) in a volume of 5 ml of serum-free DMEM™ containing 0.1 mM cloroquine and 600 µg/ml DEAE Dextran™, and cells were incubated for 3-3.5 h at 37°C. Transfected cells were then briefly treated (approximately 2 min) with 10% dimethyl sulfoxide in PBS and incubated at 37°C for 16-24 h in DMEM™ containing 10% FCS. At 24 h after transfection, culture medium was removed and replaced with serum-free DMEM™ (6 ml/dish). Incubation was continued for 3 days at 37 °C, at which time the spent medium was collected and fresh serum-free medium was added. After an additional 3 days at 37°C, the spent medium was again collected and cells were discarded.

CHO cells expressing CD28, CD5 or B7 were isolated as described by Linsley et al., (1991) supra, as follows: Briefly, stable transfectants expressing CD28, CD5, or B7, were isolated following cotransfection of dihydrofolate reductase-deficient Chinese hamster ovary (dhfr⁻ CHO) cells with a mixture of the appropriate expression plasmid and the selectable marker, pSV2dhfr, as described above in Example 1. Transfectants were

then grown in increasing concentrations of methotrexate to a final level of 1 μM and were maintained in DMEM™ supplemented with 10% fetal bovine serum (FBS), 0.2 mM proline and 1 μM methotrexate. CHO lines expressing high levels of CD28 (CD28$^+$ CHO) or B7 (B7$^+$ CHO) were isolated by multiple rounds of fluorescence-activated cell sorting (FACS^R) following indirect immunostaining with mAbs 9.3 or BB-1. Amplified CHO cells negative for surface expression of CD28 or B7 (dhfr$^+$ CHO) were also isolated by FACS^R from CD28-transfected populations.--

On page 56, line 33, please replace the paragraph that begins "Immunostaining and FACS^R Analysis," and continues through page 57, line 12, with the following:

-- Immunostaining and FACS^R Analysis. Transfected CHO cells or activated T cells were analyzed by indirect immunostaining. Before staining, CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with murine mAbs 9.3 (Hansen et al., Immunogenetics 10:247 (1980)) or BB-1 (Yokochi et al., supra) at 10 μg/ml, or with Ig fusion proteins (CD28Ig, B7Ig, CD5Ig or chimeric mAb L6 containing Ig Cγ1, all at 10 μg/ml in DMEM™ containing 10% FCS) for 1-2 h at 4°C. Cells were then washed, and incubated for an additional 0.5-2h at 4°C with a FITC-conjugated second step reagent (goat anti-mouse Ig serum for murine mAbs, or goat anti-human Ig Cγ serum for fusion proteins (Tago, Inc., Burlingame, CA). Fluorescence was analyzed on 10,000 stained cells using a FACS IV^R cell sorter (Becton Dickinson and Co., Mountain View, CA) equipped with a four decade logarithmic amplifier.--

On page 58, line 1, please replace the paragraph beginning "SDS Page" with the following:

-- SDS Page. SDS-PAGE was performed on linear acrylamide gradients gels with stacking gels of acrylamide. Aliquots (1 μg) B7Ig (lanes 1 and 3 of Figure 10) or CD28Ig (lanes 2 and 4) were subjected to SDS-PAGE (4-12% acrylamide gradient) under nonreducing (-βME, lanes 1 and 2) or reducing (+βME, lanes 3 and 4) conditions. Lane 5

of Figure 10 shows molecular weight (M_r) markers. Gels were stained with Coomassie Brilliant Blue, destained, and photographed or dried and exposed to X-ray film (Kodak™ XAR-5; Eastman Kodak Co., Rochester, NY) for autoradiography to visualize proteins.--

Please replace the three sequential paragraphs starting on page 59, line 27, with "Radiolabeling of B7Ig," and continuing to page 61, line 8, with the following three paragraphs:

-- Radiolabeling of B7Ig. Purified B7Ig (25μg) in a volume of 0.25 ml of 0.12 M sodium phosphate, pH 6.8 was iodinated using 2 mCi ^{125}I and 10 μg of chloramin T™. After 5 min at 23°C, the reaction was stopped by the addition of 20 μg sodium metabisulfite, followed by 3 mg of KI and 1 mg of BSA. Iodinated protein was separated from untreated ^{125}I by chromatography on a 5-ml column of Sephadex™ G-10 equilibrated with PBS containing 10% FCS. Peak fractions were collected and pooled. The specific activity of ^{125}I-B7Ig labeled in this fashion was 1.5 X 10^6 cpm/pmol.

B7Ig was also metabolically labeled with [^{35}S]methionine. COS cells were transfected with a plasmid encoding B7Ig as described above. At 24 h after transfection, [^{35}S]methionine (<800 Ci/mmol; Amersham Corp., Arlington Heights, IL) was added to concentrations of 115 μCi/ml) in DMEM™ containing 10% FCS and 10% normal levels of methionine. After incubation at 37°C for 3 d, medium was collected and used for purification of B7Ig as described above. Concentrations of [^{35}S]methionine-labeled B7Ig were estimated by comparison of staining intensity after SDS-PAGE with intensities of known amounts of unlabeled B7Ig. The specific activity of [^{35}S]methionine-labeled B7Ig was approximately 2 x 10^6 cpm/μg.

Binding Assays. For assays using immobilized CD28Ig, 96-well plastic dishes were coated for 16-24 h with a solution containing CD28Ig (0.5 μg in a volume of 0.05 ml of 10 mM Tris, pH 8). Wells were then blocked with binding buffer (DMEM™ containing 50 mM BES, pH 6.8, 0.1% BSA, and 10% FCS) (Sigma Chemical Co., St. Louis, MO)

before addition of a solution (0.09 ml) containing ^{125}I-B7Ig (3 x 10^6 cpm, 2 X 10^6 cpm/pmol) or [^{35}S]-B7Ig (1.5 x 10^5 cpm) in the presence of absence of competitor to a concentration of 24 nM in the presence of the concentrations of unlabeled chimeric L6 mAb, mAb 9.3, mAb BB-1 or B7Ig, as indicated in Figure 12. After incubation for 2-3 h at 23°C, wells were washed once with binding buffer, and four times with PBS. Plate-bound radioactivity was then solubilized by addition of 0.5 N NaOH, and quantified by liquid scintillation or gamma counting. In Figure 12, radioactivity is expressed as a percentage of radioactivity bound to wells treated without competitor (7,800 cpm). Each point represents the mean of duplicate determinations; replicates generally varied from the mean by ≤ 20%. Concentrations were calculated based on a M_r of 75,000 per binding site for mAbs and 51,000 per binding site for B7Ig. When binding of ^{125}I-B7 to CD28$^+$ CHO cells was measured, cells were seeded (2.5 x 10^4/well) in 96-well plates 16-24 h before the start of the experiment. Binding was otherwise measured as described above.--

On page 62, line 11, please replace the paragraph beginning "Cell separation and Stimulation" with the following:

-- Cell [s]Separation and Stimulation. PBL were isolated by centrifugation through Lymphocyte Separation Medium™ (Litton Bionctics, Kensington, MD) and cultured in 96-well, flat-bottomed plates (4 x 10^4 cells/well, in a volume of 0.2 ml) in RPMI™ containing 10% FCS. Cellular proliferation of quadruplicate cultures was measured by uptake of [^{3}H]thymidine during the last 5 h of a 3 day (d) culture. PHA-activated T cells were prepared by culturing PBL with 1 µg/ml PHA (Wellcome) for 5 d, and 1 d in medium lacking PHA. Viable cells were collected by sedimentation through Lymphocyte Separation Medium™ before use.--

On page 69, line 1, please replace the paragraph beginning "RNA was prepared" with the following:

-- RNA was prepared from stimulated PHA blasts by the procedure described by Chomczynki and Sacchi, Anal. Biochem. 162:156 (1987), incorporated by reference

herein. Aliquots of RNA (20 μg) were fractionated on formaldehyde agarose gels and then transferred to nitrocellulose by capillary action. RNA was crosslinked to the membrane by UV light in a Stratalinker™ (Stratagene, San Diego, CA), and the blot was prehybridized and hybridized with a ^{32}P-labeled probe for human IL-2 (prepared from an approximately 600-bp cDNA fragment provided by Dr. S. Gillis; Immunex Corp., Seattle, WA). Equal loading of RNA samples was verified both by rRNA staining and by hybridization with a rat glyceraldehyde-6-phosphate dehydrogenase probe (GAPDH, an approximately 1.2-kb cDNA fragment provided by Dr. A. Purchio, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA).--

In the Claims:

Please cancel claims 77-86 without prejudice as directed to a non-elected invention.

Please amend claim 87 as follows.

--87. (amended) A method for [generating and identifying] producing an antibody[ies] or fragment thereof directed against [for] a B7 antigen having SEQ ID NO. 8 or a fragment of SEQ ID NO. 8, which antibody reacts with B7 on B7 positive B cells to [that] inhibit B cell[s from binding] interaction with CD28, comprising:

(a) [screening a sample of B cells for B cells that bind CD28;

(b) isolating and purifying proteins mediating the B cell binding with CD28;

(c) immunizing an animal with an antigenic portion of the purified proteins] using the B7 antigen or a fragment thereof so as to produce antibodies that react with B7 antigen or a fragment thereof;

[(d) harvesting antibodies so produced;] and

(d) [b] screening the antibodies so prepared for an antibody[ies] that reacts with the B7 or fragment thereof on B7 positive B cells and inhibits B cell interaction with CD28 [binding to B cells].--

Please add new claims 89-93 as follows:

--89. (new) The method of claim 87, wherein the B7 antigen or fragment is B7Ig.--

--90. (new) A method for producing an antibody or fragment thereof directed against a B7 antigen having SEQ ID NO. 8 or a fragment of SEQ ID NO. 8, which antibody reacts with B7 on B7 positive B cells to inhibit B cell interaction with CD28, comprising:

(a) using a B7 fusion protein having SEQ ID NO. 8 or a fragment of SEQ ID NO. 8 so as to produce antibodies that react with the B7 or a fragment thereof; and

(b) screening the antibodies so prepared for an antibody that reacts with the B7 antigen or a fragment thereof on B7 positive B cells and inhibits B cell interaction with CD28.--

--91. (new) The method of claim 90, wherein the B7 fusion protein is B7Ig.—

--92. (new) The method of claim 91, wherein the B7Ig is encoded by DNA as deposited with the American Type Culture Collection (ATCC) as accession No. 68627.

--93. (new) An antibody produced by the method of claim 87 or 90.--

In the Abstract:

In accordance with 37 C.F.R. §§1.121(b)(1)(i)-(ii), please delete the abstract, beginning on page 89, lines 4-17 of the application and replace it with the following:

--The invention [identifies the B7 antigen as a ligand that is reactive with the CD28 receptor on T cells. Fragments and derivatives of the B7 antigen and CD28 receptor, including fusion proteins having amino acid sequences corresponding to the extracellular domains of B7 or CD28 joined to amino acid sequences encoding portions of human immunoglobulin Cγ1, are described. Methods are provided for using B7 antigen, its fragments and derivatives, and the CD28 receptor, its fragments and derivatives, as well as antibodies and other molecules reactive with B7 antigen and/or the CD28 receptor, to regulate CD28 positive T cell responses, and immune responses mediated by T cells. The invention also includes an assay method for detecting ligands reactive with cellular receptors mediating intercellular adhesion] provides a method for generating and identifying antibodies directed against a B7 antigen having SEQ ID NO. 8 or a fragment of SEQ ID NO. 8, which antibodies inhibit B cells from binding CD28, comprising immunizing an animal with the B7 antigen so as to produce the antibodies; and screening the antibodies for antibodies that bind B7 and inhibit CD28 binding to B cells.--